(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 128 249**
B1

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: **03.08.88**

(21) Application number: **83303060.4**

(22) Date of filing: **27.05.83**

(51) Int. Cl.⁴: **C 07 K 5/06**, C 07 K 5/08, C 07 K 5/10, C 07 K 7/06, C 07 K 1/06, A 61 K 37/02

(54) **Antimicrobial disulfide prodrugs.**

(30) Priority: **08.06.82 US 386365**

(43) Date of publication of application:
19.12.84 Bulletin 84/51

(45) Publication of the grant of the patent:
03.08.88 Bulletin 88/31

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

(56) References cited:
US-A-4 237 267
US-A-4 258 193
US-A-4 284 624
US-A-4 325 943
US-A-4 325 944
US-A-4 325 945

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EP 0 128 249 B1

Courier Press, Leamington Spa, England.

Description

This invention relates to antimicrobial compositions containing, as active ingredients, prodrugs whose structures have a known mercapto-containing antimicrobial residue attached to an oligopeptide chain by means of a disulfide link. Further, this invention conerns compounds for use in therapy by producing antimicrobial activity from such prodrugs together with certain new chemical compounds, also useful for using this invention.

Background of the Invention

The prior art recognizes that peptide transport permease systems are one mechanism by which chemical substances are carried through the cell membrane of an antimicrobial organism. Both di- and oligopeptide transport systems are present in the cell membrane, for example, in the cell membrane of Escherichia coli, B. C. Ames, Proc. Nat. Acad. Sci. VSA *70* 456 (1973), C. Gilvarg, Nature, the New Biology *241* 161 (1973).

Peptide transport systems are widespread in both procaryotic and eukaryotic microorganisms. A prodrug which can be transported per se through the cell membrane of such organisms via the permease system and, then, release the drug within the cell would possess enhanced activity.

A number of synthetic derivatives have been prepared which take advantage of these transport systems such as those described by M. M. Ponpipom, et al., J. Med. Chem. *24* 1388 (1981), European Patent Office application No. 38,541 or C. Philip et al., PCT application, publication No. WO81/01145. Some of these types of compounds were designed to limit toxicity or to achieve more specific biological activity. Most compounds of the prior art are active, without degradation at the receptor site, in the transport form due to their resistance to intracellular peptidases. In other words, they are not in latentiated form as are the compounds of this invention. For example, the Philip publication, cited above, discloses anti-tumor moieties which are attached by a covalent bond to a polypeptide.

A number of potentially useful chemotherapeutic agents are present in the prior art which are impermeant or poorly permeant to the cell membrane of an infecting organism. The impermeant nature of these compounds may be due to the inherent physico-chemical properties of the compounds or due to an acquired resistance to them in the permease system of the cell membrane of the target species.

Several related United States patents describe compounds which have angiotensin converting enzyme inhibiting activity whose structures have a tripeptide containing a central cysteinyl unit attached to a proline-like ring by means of a disulfide bond, U.S. Patent Nos. 4,284,624; 4,325,943; 4,325,944 and 4,325,945.

Another series of U.S. patents, U.S. Patent Nos. 4,237,267 and 4,258,193, disclose a large number of disulfides which are used in exchange reactions, including a few compounds with structures having pyridine N-oxide attached via a disulfide bond to the alanyl unit of an oligopeptide or to alanine itself.

The S-ethylthio protective group has been used, along with others, in procedures to prepare cysteine containing peptides. The S-ethylthio group was inserted into the peptide structures by displacement of an S-guanylthio moiety by ethylmercaptan in a solvent system of dimethylformamide-triethylamine, H. Kunzek et al., J. Prakt. Chemie, *322* 186 (1980).

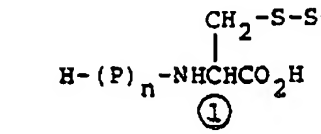
J. V. Castell et al., Helv. *62* 2507 (1979), reported that a mixed cysteine-pyridine disulfide reacted, by displacement, with various mercaptans in acetic acid to form mixed disulfides during a study of the use of 2-pyridine sulfenyl chloride to form protected cysteinyl derivatives.

Description of the Invention

This invention concerns, as active antimicrobial ingredients, a series of prodrugs whose antibacterial or antifungal activities are enhanced or whose drug delivery capabilities are improved. The active warhead is attached via a disulfide bridge to a specific di- or oligopeptide chain. When the prodrug is placed in contact with a fungal or bacterial target species, the oligopeptide chain enhances absorption of the warhead, or antimicrobial residue, through the peptide transport channels of the cell membrane of the infecting species. Then, within the cell, the prodrug reacts with intracellular sulfhydryl containing compounds, such as glutathione, which are known to be present in the cells to release the active warhead by a disulfide exchange reaction.

The active anti-microbial prodrugs, which are the basis of this invention, must have structures that possess a number of features necessary for them to act as prodrugs having improved properties. The structures, usually, contain an oligopeptide backbone of from 2—6 amino acid units, one of which is, necessarily, a L-alanyl which is β-substituted through a disulfide link with a residue of a known mercaptan antimicrobial agent. This L-alanyl unit is designated as the "carrying" unit of the peptide backbone. The peptide chain must have a free amino group at the N- or amino- terminus, preferably, also a free carboxy group at the C- or carboxy- terminus.

The active ingredients of this invention are exemplified by the following structural formula:

I 

in which:

n is an integer of from 1—5;

P is, individually, alanyl, ornithyl, lysyl or phenylalanyl; and

W is the residue of a compound having antimicrobial activity whose structure contains a mercaptan group ((—SH).

The configuration of the C-unit amino acid (1) must be L. The configuration of the adjacent amino acid unit (2) must, likewise, be L. More removed units at positions 3—6 of the backbone oligopeptide may be either D or L. It should be emphasized that, for convenience, the description of this invention numbers the warhead-carrying amino acid unit as 1 at the carboxy or C-unit of the oligopeptide chain, with numbering proceeding down the chain toward the amino or N terminus. In fact, the carrying unit need not be at a terminal position of the oligopeptide backbone.

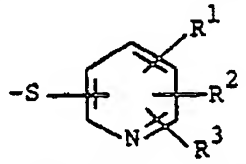
More specifically, W is the residue of any antibacterial or antifungal agent whose structure contains a free mercapto radical (—SH). This group of compounds is well known in the art, for example, in European Patent Office application No. 38,541 referred to above, at page 2 line 5 to page 3 line 6. The impermeant or poorly permeant properties of many of the mercaptan containing therapeutic agents is also disclosed in the EPO reference.

The warhead parent compounds (HSW) may range from topical agents such as the 2-mercaptopyridines or alkylmercaptans to systemic agents such as the known 4-[N-(2-mercaptoethyl)]-aminopyridine-2,6-dicarboxylic acid or other cell wall active antibacterials.

The pyridine containing warheads are preferred, for example, those in the literature such as U.S. Patent Nos. 2,540,218; 3,590,035; 3,700,676, 3,759,932; 3,773,770; 3,968,188; 3,972,888 and German Offen. 2,165,752 (CA *77* 126557j).

Species of such compounds are 2-pyridinethiol-N-oxide (pyrithione), 3-methyl-2-pyridinethiol-N-oxide, 3-ethoxy-2-pyridinethiol-N-oxide, 3,5-dichloro-2-mercaptopyridine-N-oxide, 5-chloro-2-mercaptopyridine-N-oxide, 5-bromo-2-mercaptopyridine-N-oxide, 3,4,5,6-tetrachloro-2-pyridinethiol-N-oxide, 6-mercapto-2-picoline-N-oxide, 4-methoxy-2-pyridinethiol-N-oxide, 5-methyl-2-pyridinethiol-N-oxide, 4-dodecylthio-2-pyridinethiol-N-oxide, 4-benzylsulfonyl-2-pyridinethiol-N-oxide, 4-benzylthio-2-pyridinethiol-N-oxide, 4,7-dimethyl-2-pyridinethiol-N-oxide, 1-hydroxy-2-pyridinethione, pyridine-2-thiol.

The pyridyl subgroup of the compounds of formula I is exemplified by the formula in which SW is:

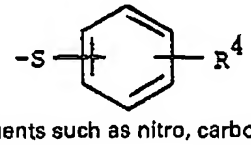
II 

in which R^1, R^2 and R^3, each, are hydrogen, alkyl of 1—12 carbons, alkoxy of 1—12 carbons, alkylthio of 1—12 carbons, phenoxy, phenylthio, phenylsulfonyl, benzyloxy, benzylthio, carboxy or halo, such as chloro or bromo; or N-oxide derivatives of said compounds.

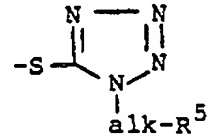
Said alkyl or alkoxy groups are preferably of 1—2 carbons.

It should be noted that the pyridylthio containing compounds, especially those having the specific pyridylthio or pyridylthio-N-oxide moieties in their structures, also serve as intermediates since these moieties are excellent leaving groups in the disulfide exchange reaction described below.

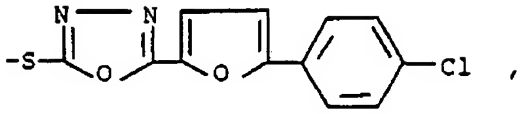
Other —SW groups of formula I are:

(a) 

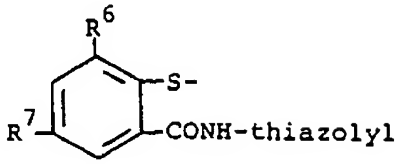
in which R^4 is one or two substituents such as nitro, carboxy, halo, cyano, lower alkyl of 1—6 carbons or lower alkoxy of 1—6 carbons;

(b) 

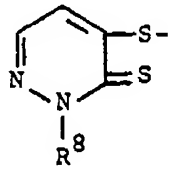
in which alk is alkylene of 2—6 carbons and R^5 is carboxy, sulfonamide, sulfamyl, carbomethoxy, carbamyl.

(c)  ,

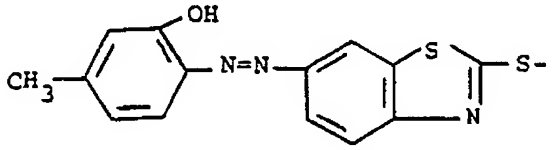
U.S. Patent No. 4,134,893;

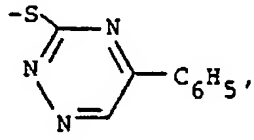
(d) $-S-CH_2CH_2OH$, CA 90 98007e;  (e)

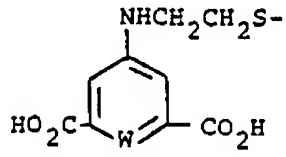
in which R^6 and R^7 are hydrogen, chloro or bromo, CA *86* 89671v;

(f) 

in which R^8 is hydrogen, methyl, ethyl, benzyl, U.K. Patent No. 2,025,416;

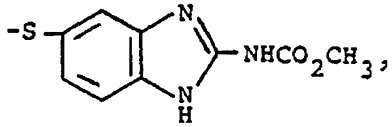
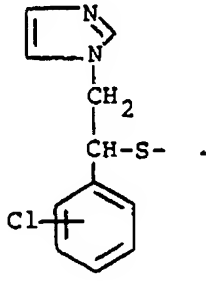
(g)  , CA 93 150163g;

(h)  , CA 94 185507c;

(i)  , (j) -S-alk

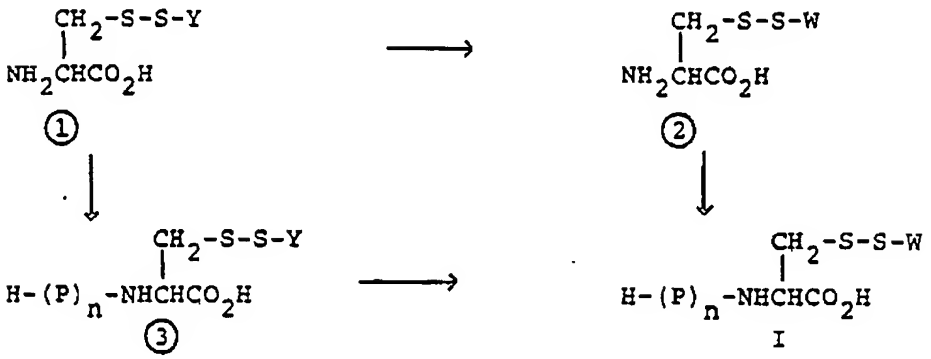
in which alk has from 1—12 carbons.

(k) $-SCH_2CO_2H$, (l) $-SCH_2\overset{O}{\overset{\|}{P}}(OH)_2$, $-SCH_2\underset{OH}{CH}CH_2\overset{O}{\overset{\|}{P}}(OH)_2$,

(p)  (q) 

The active ingredients of formula I are prepared by the following reaction sequence:

Sequence A



In reaction Sequence A, n, P and W are as defined above for formula I. Y is a chemically displaceable or leaving group, when taken with the adjacent S; for example, pyridyl, pyridyl-N-oxide, and guanyl.

The key reaction is a disulfide exchange between a mixed disulfide and the warehead mercaptan (HSW). The mixed disulfide has a readily displaceable thio containing group (—S—Y) S-connected to the β-alanyl unit itself (1) or to the oligopeptide containing the β-alanyl unit (3). The reaction is most cnveniently run on the amphoteric form of the amino acid or oligopeptide starting material. One skilled in the art will recognise that the reaction can, also, be run on disulfide starting materials having amino or carboxy protecting groups on the oligopeptide chain or on the warhead. These are then removed after the sulfur-sulfur interchange.

The reaction is carried out by combining the mixed disulfide (1 or 3) with a stoichiometric quantity or, preferably, an excess of the warhead mercaptan (HSW) in a solvent in which both are substantially soluble, for example, an organic solvent such as dimethylformamide, dimethylacetamide, dimethylsulfoxide, ethers such as dioxane, ethyl ether or tetrahydrofuran, halogenated solvents such as methylene chloride, chloroform or carbon tetrachloride or esters such as ethyl acetate. Aqueous solvents, preferably at an alkaline pH, can be used if the warhead mercaptan is water soluble.

The course of the reaction is followed by thin layer chromatography using methods described in the working examples. Ambient or room temperature of reaction until substantial completion, from 30 minutes to 12 hours, is very satisfactory. Moderate heat can be, optionally, used for sluggish interchanges. Therefore, in fact, the exchange reaction can be run at temperature chosen from −15° up to about 100°. The desired chemical products of the reaction are isolated by standard means, after removal of any protective groups. Purification by gel filtration over a bead-formed gel prepared by cross-linking dextran with epichlorohydrin ("Sephadex", registered Trade Mark, Pharmacia) is convenient.

The antimicrobial compositions of this invention comprise two sub-groups of dosage units which are used to treat bacterial or fungal infections. The surface infections are the particular targets of the active compounds of formula I. Especially preferred are the active ingredients in which the warhead portion of the oligopeptide prodrug is derived from a pyridylthio, a thiophenol or an alkyl mercaptan, each group of which are known to the art to have antimicrobial properties. These are not usually used systemically in the prior art because of their toxicity or physico-chemical properties.

The prodrugs of this invention are brought into contact with the infectious species by use of carrier forms such as solutions, emulsions or suspensions for topical use, shampoos, creams, troches, gums, drenches, soaps, dry or wet pressure sprays, bandages, suppositories, powders, and mouth washes. These are prepared as described in Remington's Pharmaceutical Sciences, 13th Edition, 1965 Mack. The concentration of the prodrug will depend on both the inherent activity of the warhead as well as that of the prodrug form which has enhanced cell membrane permeability, taken with the product form and the site of infection. Generally speaking, for topical use, quantities not toxic to the patient but having effective antimicrobial activity are chosen from the range of 0.2—10%, preferably 0.5—4%, by weight.

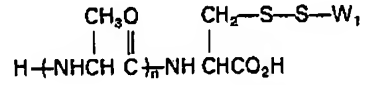
In addition, the prodrugs of this invention which have efficacy against systemic infections are combined in oral and parenteral dosage units in quantities which are effective against the infecting agents but which are nontoxic to the patient. Parenteral use includes intravenous, intramuscular or infusion administration.

The infectious microbes which are the targets of the prodrug active ingredients of this invention, are any that are known to be susceptible to the warhead, in vivo, as well as those against which the novel increased cell membrane permeability, newly discovered here, makes the warhead active in a practical way. Examples of such organisms are Escherichia coli, Aspergillus niger, Chetonium globossum, Staphylococcus aureus, Candida albicans, Microsporum canis, Peptococcus acnes, Peptococcus

granulosum, Peptococcus saccherolyticus, various Helminthosporums, Trichophyton rubrum, Candida tropicalis or Cryptococcus neoformans.

This invention comprises the antimicrobial compositions, described above for the therapeutic use on a human or animal subject or patient infected with a bacterium or fungus, either topically, orally or parenterally, in a quantity which is nontoxic to the subject but effective against the infecting bacterium or fungus.

Also, part of this invention are new chemical compounds for use as chemical intermediates and topical antimicrobial agents having the structural formula:



III

in which:

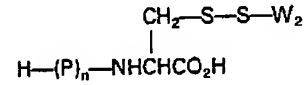
n is an integer of from 1—5;

W_1 is as defined for structural formula II (W) above, and each of the amino acid units of the oligopeptide chain has the L-configuration.

Preferred compounds of this subgroup are those of formula III in which n is 1—3. Another such group have structures where W_1 is 2-pyridyl, 2-pyridyl-1-one, methyl-2-pyridyl or methyl-2-pyridyl-1-one.

Advantageous compounds of this invention are those of structural formula III and the preferred group in which n is 1.

A second group of new chemical compounds of this invention are those having the structural formula:



IV

in which:

n and P are as defined for formula I above;

W_2 is 2,6-dicarboxy-pyridyl-4-aminoethyl;

and each of the amino acid units of the oligopeptide chain is of the L-configuration.

The reactions, outlined above, are described to be carried out in liquid phase, however, as with most peptide preactions, solid phase or enzyme technology may be used on certain of these as will be known to the art, R. B. Merrifield, Biology *3* 1385 (1964) or J. Am. Chem. Soc, *85* 2149 (1963).

Also, one skilled in the art will recognize that any groups present in the peptide chain or in the warhead W which may be chemically reactive under the conditions of reaction sequence A or the earlier reactions should be protected, as known to the art, U.S. Patent No. 3,803,120 or EPO Application No. 38,541 or U.S. Patent No. 3,957,803.

While the prodrugs of this invention are most useful in treating bacteria or fungus microbes topically, or even systemically in a whole animal, the same prodrug concept can be used for other purposes. If the warhead, W, is a known compound having other pharmacodynamic properties, the oligopeptide prodrug can be used to increase cell membrane permeability or to improve distribution or absorption of drug. In other instances, the oligopeptide prodrug can be used to prepare new pharmaceutical forms such as injectable preparations of compounds not readily useful for such uses because of their physico-chemical properties such as low solubility. These utilities may be applied to other mercaptan containing drugs such as penicillamine, thiopental, propylthiourea, 6-mercaptopurineriboside, 6-thioguanine, 6-mercaptopurine, N-acetylcysteine or N-(2-methyl-3-thiopropionyl)-proline.

The prodrugs of formula I are represented herein as their amphoteric polypeptide forms. One skilled in the art will recognize that salt forms of the prodrugs may be equally useful, such as pharmaceutically acceptable acid addition salts when a basic center is present in the polypeptide chain or in the carried warhead. Altenatively, pharmaceutically acceptable basic salts derived from the usual bases, such as those having alkali metal or nontoxic organic amine cations, can be prepared if an acid center is present. Both types of salts are prepared as known to the art, usually by contacting the prodrug with an excess of acid or base in a suitable solvent.

The following examples are designed to teach the practice of this invention as well as the biological activity of representative compounds of this invention. All temperature are in degrees Centigrade. T.l.c. refers to thin layer chromatography. NMR refers to nuclear magnetic resonance spectrum. MPLC refers to medium pressure liquid chromatography.

Example 1

S-Ethylthiocysteine (497 mg, 2.75 mm, m.p. 180°, lit. 199—200°) was dissolved in 10 ml of water, followed by 270 mg (2.5 mm) of sodium bicarbonate. The mixture was cooled to 0°, at which temperature, 2.5 ml of 1N sodium hydroxide solution was added, along with 6 ml of acetonitrile and a solution of 380 mg (3.3 mm) of alanine N-carboxyanhydride (Leuch's anhydride, *The Peptides*, E. Grossand and J. Meienhofer, Academic Press, page 85) in 2 ml of acetonitrile. After standing at room temperature for 3 hours, the pH was adjusted to 5.5 with 3N hydrochloric acid. The mixture was evaporated to give an impure solid which was treated with water to separate the water soluble material which was purified by medium pressure

reverse phase chromatography over silica gel, eluting, first, with water followed by 5% methanol, 10% methanol and 25% methanol. The four fractions were homogeneous by thin layer chromatography. They were combined and lyophilized to give 0.35 g (50%) of L-alanyl-S-ethylthiocysteine; NMR spectrum (D_2O + 1 drop DCl, ppn) 3.2(t), 2.8(g), 1.6(d), 1.3(t).

Anal. Calcd. for $C_8H_{16}O_3N_2O_3S_2 \cdot 1/2H_2O$: C, 36.77; H, 6.56; N, 10.72. Found: C, 37.14; H, 6.56; N, 10.72.

Example 2

A mixture of 3.02 g of L-cysteine and 75 ml of glacial acetic acid was added slowly to a mixture of 11.0 g of 2,2'-dipyridyldisulfide and 75 ml of glacial acetic acid. The precipitated solid was removed. The filtrate was diluted with 600 ml of ethyl ether to separate 3.7 g of β-L-alanyl 2-pyridyl disulfide.

Phosgene was passed through a mixture of 0.5 g of L-alanine and 20 ml of dry tetrahydrofuran with stirring and refluxing. After 2 hours, the clear solution was cooled and concentrated. The residue, 0.41 g of alanyl N-carboxyanhydride, was recrystallized from toluene.

A mixture of 0.68 g (2.97 mm) of the disulfide, 0.30 g of sodium carbonate, 3.0 ml of 1N sodium hydroxide solution, 12 ml of water and 12 ml of acetonitrile was cooled to −10°. An excess of alanyl N-carboxyanhydride was added, dissolved in 7 ml of acetonitrile. The mixture was stirred at 0° for 3 hours. The liquid phases were separated. The aqueous layer was taken to pH 7 with a few drops of sulfuric acid. Ethanol was added. Sodium sulfate was removed by filtration. The filtrate was evaporated. Ethanol was added and the resulting solid was 0.194 g of L-alanyl-β-L-alanyl 2-pyridyl disulfide which was purified twice over a reverse phase column as described above, with elution, first with water, 10% methanol and 50% methanol, and, then, the second time with 50% methanol to give 23 mg of product which was pure by thin layer chromatography.

Anal. Calcd for $C_{11}H_{14}N_3O_3S_2 \cdot Na \cdot 3/4H_2O$: C, 39.22; H, 4.64; N, 12.47. Found: C, 39.03; H, 4.98; N, 12.27.

Example 3

A mixture of 0.47 g (1.91 mm) of L-alanyl N-oxy-2-pyridyl disulfide, prepared as in Example 2 using pyrithione as the mercapto starting material, 0.191 g of sodium carbonate, 1.91 ml of 1N sodium hydroxide solution, 8 ml of water and 8 ml of acetonitrile at 0° was reacted for 3 hours with 0.26 g (2.3 mm); of L-alanyl-N-carboxyanhydride dissolved in acetonitrile. The mixture was worked up as described in Example 2 using thin layer chromatography (t.l.c.) over cellulose plates with methanol-saline as developing solvents and ninhydrin or ultraviolet light as markers for following the reaction. Purification was by gel filtration ("Sephadex" G—10) and, then, by a reverse phase medium pressure cellulose column to give 37 mg of the desired, L-alanyl-β-L-alanyl N-oxy-2-pyridyl disulfide.

Anal. calcd. for $C_{11}H_{15}N_3O_4S_2 \cdot 2H_2O$: C, 37.38; H, 5.42; N, 11.89. Found: C, 37.06; H, 4.99; N, 11.50.

This material (2 g) is mixed with 10 g of titanium oxide, 1 g of oxide coloring agents, 7 g of oxyethylenated stearyl alcohol and 6 g of polyglycol stearate, with water added to make 100 g of 2% cream which is applied to the infected body surface.

Example 4

A solution of 0.77 g (6.7 mm) of L-alanyl-N-carboxy anhydride in 10 ml of acetonitrile was added at −10°, with stirring to a mixture of 2.0 g (6.1 mm) of β-L-alanyl 2-pyridyl disulfide, prepared as in Example 2, 0.61 g of sodium carbonate, 6.1 ml of 1N sodium hydroxide, 25 ml of water and 31 ml of acetonitrile. The reaction was continued for two hours in the cold. The phases were separated. The aqueous phase was washed once with 20 ml of cold acetonitrile and, then, heated to 40° for 5 minutes.

The resulting solution was mixed with 12 ml of water, 31 ml of acetonitrile and 2.45 ml of 1N sodium hydroxide (pH ~9). After cooling to −10°, a solution of 0.77 g (6.7 mm) of L-alanyl-N-carboxyanhydride in 10 ml of acetonitrile was added. The resulting mixture was reacted in the cold for 2 hours. The aqueous layer was washed with cold acetonitrile, then, adjusted to pH 5.85 with sulfuric acid. Ethanol was added to separate inorganic salts. The filtrate was evaporated to leave a residue which was dissolved in 10 ml of water which solution was, again, diluted with ethanol. The filtrate was concentrated. The residue was taken up in water and lyophilized to give 1.05 g of product.

This material (200 mg) was separated into three products using reverse phase medium pressure liquid chromatography over a cellulose column using 1:1 5% salt solution:methanol. In order of elution, the dipeptide (43 mg), tripeptide (19 mg) and tetrapeptide (11 mg) were obtained. The separation was repeated on 0.82 mg of mixture to give analytical samples:

(a) L-alanyl-β-L-alanyl 2-pyridyl disulfide identical to that previously described.

(b) L-alanyl-L-alanyl-β-L-alanyl 2 pyridyl disulfide

Anal. calcd. for $C_{14}H_{20}N_4O_4S_2 \cdot H_2O$: c, 43.06; H, 5.68; n, 14.34. Found: C, 42.88, H, 5.60; N, 14.51.

(c) L-alanyl-L-alanyl-L-alanyl-β-L-alanyl 2-pyridyl disulfide

Anal. Calcd. for $C_{17}H_{25}N_5O_6S_2 \cdot 1/2H_2O$: C, 45.12; H, 5.79; N, 15.47. Found: C, 45.43; H, 5.78; N, 14.84.

Example 5

A mixture of 3.0 g (11.2 mm) of 5-thioguanylcysteine and 50 ml of dry dimethylformamide was cooled to −15°, then, mixtures of 1.11 g (10 mm) of 2-mercaptopyridine and 15 ml of dimethylformamide as well as 3.03 g (30 mm) of triethylamine in 15 ml of dimethylformamide were added, separately, with stirring.

The mixture was allowed to warm to room temperature and was stirred for 1 hour. The mixture was filtered. The solid residue was washed with methylene chloride, methanol and ether to give 1.12 g (43%) of β-L-alanyl 2-pyridyl disulfide. This material (500 mg) is reacted with D,L-phenylglycyl-N-carboxyanhydride, as described above, to give D,L-phenylglycyl-β-L-alanyl 2-pyridyl disulfide.

Example 6

A mixture of 0.83 g (2.5 mm) of di-*tert.*-boc-ornithine, 0.54 g (2.5 mm) of N,N'-disuccinamidyl carbonate [H. Ogura, Tetrahedron Letters *49* 4745 (1969)], 0.20 g (2.5 mm) of pyridine and 15 ml of acetonitrile was stirred overnight at room temperature. The solvent was evaporated. The residue was dissolved in ethyl acetate which extract was washed with water, brine, then concentrated, after drying, to give 0.98 g (91%) of di-*tert.*-boc-ornithine, N-hydroxysuccinimide ester.

The activated ester (1.87 g, 4.35 mm) in 26 ml of dioxane was added to another mixture of 2.06 g (4.79 mm) of β-L-alanyl 2-pyridyl disulfide hydrochloride, 1.10 (13.05 mm) of sodium carbonate and 22 ml of water. The resulting mixture was stirred at room temperature for 5 hours. The solvent was evaporated. The residue was dissolved in water (pH ~8.3) and the extract washed with ethyl acetate. The aqueous layer was taken to pH 3.5 with 10% citric acid, then, extracted with ethyl acetate. The acid phase layer was washed with citric acid solution and brine, then, dried and concentrated in vacuo to give 1.83 g of residue which demonstrate the desired product plus impurities by t.l.c. analysis carried out as described above. The residue was purified over a silica column by medium pressure liquid chromatography using methylene chloride, 2% methanol in chloroform and 5% methanol in methylene chloride. The desired product was eluted by the last solvent system (0.742 g). After a further purification over a reverse phase column, 0.60 mg of di-*tert.*-butoxycarbonyl-L-ornithyl-β-L-alanyl 2-pyridyldisulfide was obtained, pure by t.l.c.

A 10 ml portion of glacial acetic acid was saturated with hydrogen bromide with cooling. The *t.*-boc-dipeptide sulfide (100 mg) was added. After stirring for 2 hours, the solvent was evaporated. The residue was triturated several times with ether, filtered and dried to give 0.098 g of L-ornithyl-β-L-alanyl 2-pyridyl sulfide, pure by t.l.c. analysis using butyl alcohol, acetic acid, water over cellulose.

Example 7

A mixture of 0.301 (1.0 mm) of L-alanyl-β-L-alanyl 2-pyridyl disulfife, prepared as in Example 4, 5 ml of water and 0.168 g (2.0 mm) of sodium bicarbonate was combined with a solution of 0.43 g (1.0 mm) of di-t.-boc-L-ornithine, N-hydroxy succinimide ester, prepared as in Example 6, in 6 ml of dioxane. Stirring the reaction mixture at room temperature for 3 hours completed the reaction, as noted by t.l.c. analysis using 1:1 5% brine/methanol. The solvent was evaporated. The residue was diluted with 13 ml of water. The mixture was cooled in an ice bath and the pH adjusted to 4 with 10% citric acid solution, while layered with ethyl acetate. The organic layer was separated and combined with two further ethyl acetate wash layers. The combined and dried organic extracts were concentrated to give 0.536 of the di-*t.*-boc-L-ornithyl-L-alanyl-β-L-alanyl 2-pyridyl disulfide. T.l.c. demonstrated one spot, using silica gel plates and a 90:10:3 methylene chloride/methanol/formic acid solvent system.

The *t.*-boc-peptide (100 mg) was combined with 1 ml of anisole and 1 ml of trifluoroacetic acid cooled in an ice bath. T.l.c., using the described procedure, indicated reaction was complete at 2 hours, with stepwise removal of the N-protective groups. The mixture was poured into 40 ml of ether with stirring. The solid was separated, washed with ether and dried to give 87 mg of L-ornithyl-L-alanyl-β-L-alanyl 2-pyridyl disulfide.

The preparation was run as described on 4 times larger scale to give 0.357 of impure tripeptide disulfide. This was dissolved in water and stirred with a weakly basic anion exchange column in free base form (IR—45). The filtrate was adjusted to pH 8.8 with 1N lithium hydroxide, then, lyophilized to give 0.416 g of L-ornithyl-L-alanyl-β-L-alanyl 2-pyridyl disulfide. This material was taken over a reverse phase medium pressure liquid chromatographic column to give the desired tripeptide.

Anal. Calcd. for $C_{16}H_{25}N_5O_4S_2 \cdot 3H_2O$: C, 40.92; H, 6.65; N, 14.91. Found: C, 40.90; H, 6.91, N, 14.80.

The reactions described above are used to prepare L-lysyl-L-alanyl-β-L-alanyl 2-pyridyl disulfide and D,L-lysyl-L-alanyl-β-L-alanyl 2-pyridyl disulfide.

A dermatological cake is prepared by mixing 2 g of L-ornithyl-L-alanyl-β-L-alanyl 2-pyridyl disulfide with 23 g of lanolin and 75 g of esters of sodium iso-thionate and fatty acids ("Igepon" A registered Trade Mark).

Example 8

L-alanyl-L-alanyl-β-L-alanyl 2-pyridyl disulfide (15.3 mm, prepared as described above) and 4-[N-2-mercaptoethyl)]amino-pyridine-2,6-dicarboxylic acid (15.8 mm) were dissolved in a solution of 0.1 M dipotassium hydrogen phosphate buffer (~4 ml). After 5 minutes at room temperature, the reaction mixture was applied to a gel column ("Sephadex" G—13, 41 × 1.3 cm) which had been equilibrated with 1.01 M acetate buffer at pH 4. The product is eluted with the same buffer. The fractions were analyzed by paper strip chromatography using the ninhydrin test. Fractions 27—42 were combined, evaporated to dryness under reduced pressure to give a residue which was dissolved in 0.2 ml of distilled water. Acetate was removed by gel filtration to give L-alanyl-L-alanyl-β-L-alanyl 2-(2,6-dicarboxy-pyridyl-4)-aminoethyl disulfide.

This material (500 mg per dosage unit) is dissolved in saline and infused into a patient in need of antimicrobial treatment.

Example 9

The R_f's of representative compounds prepared by the methods described above were determined by descending chromatography on Whatman no. 1 paper using butyl alcohol/water/acetic acid (4:1:4 upper layer) as solvent with staining by ninhydrin and Zahn's reagent to give the following results:

Peptide	2-mercaptopyridine	N-[4-(2-mercaptoethyl]-amino-pyridine-2,6-dicarboxylic acid
L-Ala-β-L-alanyl-S-	0.7	0.15
L-Ala-L-ala-β-L-ala-S-	0.6	0.21
L-Orn-L-ala-β-L-ala-S-	0.47	0.13

Example 10

Following is a general procedure for preparing the mixed oligopeptide warhead disulfides which are the active ingredients of this invention:

A solution of L-alanyl-β-L-alanyl 2-pyridyl disulfide (1 mm) in 30 ml of dimethylformamide is cooled to −15°, under a stream of nitrogen. To this solution is added a solution containing the selected mercaptan, WSH, (1 mm), triethyl amine (1 mm) and 10 ml of dimethylformamide. After the addition is complete, the reaction mixture is stirred at −15° for 30 minutes and for 60 minutes at room temperature. The reaction mixture is concentrated. The residue is dissolved in water and the solution is, then, extracted with ethyl acetate. The aqueous phase is concentrated to leave the desired product which is purified by either reverse phase medium pressure chromatography using a C_{18} modified silica gel column and a 20% aqueous methanol eluent or by passage down a gel column ("Sephadex" G—10 registered Trade Mark) as described above. Each of the mercaptans listed above is used in this procedure to prepare the selected disulfide prodrug.

Example 11

In addition to the antimicrobial compositions described above, the following methods are used:

Percentages are by weight:

A. Clear solution:

Water	97.5%
Disulfide	2.5%

B. Powder for solution:

100 Ml of a solution of the selected disulfide in saline (3%) is lyophilized to give a solid which is analyzed and placed in a multidose vial for reconstitution.

C. Powder:

Talc	99 g
Glyceryl oleate	3 g
Isopropyl nyristate	7 g
Disulfide	3 g
Perfume	2 cc

D. Suppository:

Cocoa Butter (2 g) is mixed with 0.05 g of disulfide. The mixture is melted and poured into a mold.

E. Tincture:

Disulfide	1%
Ethanol	20%
Propylene glycol	10%
Water	69%

Antimicrobial Example

A. Disc assay for inhibition by various compounds on seeded agar plates.

The medium was that of David and Mingioli, supplemented with 4 µg/ml of thiamine and 50 µg/ml of required amino acid supplements with 1.5% agar for solid media. Seeded agar plates contained 0.1 ml of

an overnight culture per 20 ml of molten agar (kept at 49°) containing appropriate supplements.

1—20 ml of test solution was added to 1/4" diameter paper discs and the discs placed on the surface of agar plates which were incubated overnight at 37°. The bacterial strains of *E. coli*, whose strains are given hereafter, were obtained from the *E. coli* Genetic Stock Center.

B. Inhibition of growth of E. coli CB64recA/F'123 by 2-mercaptopyridione (2-MP) and peptide synthons.

Zones of inhibition on seeded agar plates after overnight incubation.

	Zone of inhibition diameter, nm		
nmol	2—MP	L-Ala-L-Ala-S-2MP	L-Ala-L-Ala-L-ala-S-2MP
40	—	—	( )
200	—	—	14
800	(10—11)	15	25

— = no inhibition
( ) = partial zone, but showing significant growth inhibition
ND = not determined

C. Inhibition of growth of E. coli CB64recA/F'123 by cysteinyl peptides containing disulphide linked 4-[N-(2-mercaptoethyl)]-amino-pyridine-2,6-dicarboxylic acid (MEPDA) via the β-carbon of the C-terminal L-alanyl unit.

Zones of inhibition on seeded agar plates after overnight incubation.

	Zones of inhibition diameter, nm		
nmol	L-Ala-L-Ala-S-MEPDA	Orn-L-Ala-S-MEPDA	L-Ala-L-Ala-L-Ala-S-MEPDA
40	—	ND	(9)
200	—	ND	16
800	—	ND	24
250—500	ND	(13)	ND

nmol	Orn-L-Ala-L-Ala-S-MEPDA
40	—
200	(10)
800	(12)
250—500	ND

D. Inhibition of growth of E. coli CB64recA/F'123 by mixtures of thialysine and tripeptides containing disulphide-linked 4-[N-(2-mercaptoethyl)]-aminopyridine-2,6-dicarboxylic acid (MEPDA).

	Zone of inhibition diameter, nm	
nmol	Orn-L-Ala-L-Ala-S-MEPDA	Orn-L-Ala-L-Ala-S-MEPDA + Thialysine*
80	—	11
160	—	14
320	—	16

nmol	L-Ala-L-Ala-L-Ala-S-MEPDA	L-Ala-L-Ala-L-Ala-S-MEPDA + Thialysine*
6	—	10
30	(11)	14
60	(15)	18

* 0.5 µg thialysine was used (a sub-inhibitory concentration)

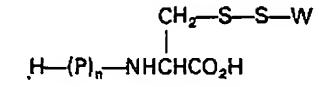
E. Fungal activity:

L-Alanyl-β-L-alanyl-1-oxo-2-pyridyldisulfide had generally less activity in vitro than did 2-mercapto-pyridyl-1-one in non-latentiated form but had advantageous physical characteristics such as a lower potential for irritation. For example, disk activity against Candida albicans β311 for the prodrug was 0.04 minimal inhibitory concentration with 0.01 for the warhead alone.

Claims for the Contracting States: BE CH DE FR GB IT LI LU NL SE

1. A 2—6 unit oligopeptide (containing a free amino group) made up of units selected from alanyl, ornithyl, lysyl and phenylalanyl, which comprises a L-alanyl unit β-substituted with a group —S—S—W wherein W is a known residue of an antimicrobial mercaptan.

2. A compound of the structural formula:



in which:

n is an integer of from 1—5;

P is, individually, alanyl, ornithyl, lysyl or phenylalanyl; and

W is a known residue of an antimicrobial mercaptan, said compound having the L-configuration at the carrying C-unit; or a salt thereof.

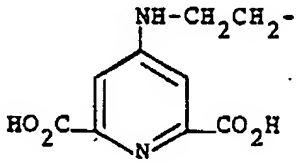
3. The compound of claim 2, in which the peptide unit adjacent to the carrying C-unit has the L-configuration.

4. The compound of claim 2 in which P is L-alanyl.

5. The compound of claim 2 in which P is L-alanyl, n is 1 and W is a pyridyl.

6. The compound of claim 2 in which P is L-alanyl, n is 1 and W is 2-pyridyl or 2-pyridyl-N-oxide.

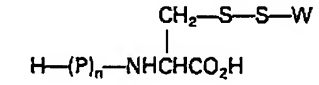
7. The compound of claim 2 in which P is L-alanyl, n is 2 and W is:



8. The compound of claim 2 in which P is L-ornithyl and n is 1.

9. The compound of claim 2 in which H-$(P)_n$— is L-ornithyl-L-alanyl.

10. A process for preparing a compound of the structural formula:



I

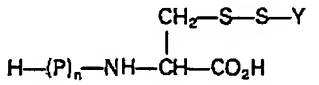
in which:

n is an integer of from 1—5;

P is, individually, alanyl, ornithyl, lysyl or phenylalanyl; and

W is a known residue of an antimicrobial mercaptan, said compound having the L-configuration at the carrying C-unit; or a salt thereof;

comprising either reacting a compound of the structural formula:



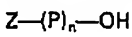
II

in which:

n is an integer of from 0—5;

P is, individually, alanyl, ornithyl, lysyl or phenylalanyl; and

Y is guanyl, pyridyl when W is other than pyridyl or pyridyl-N-oxide when W is other than pyridyl-N-oxide, with a compound of the formula, H—S—W, in which W is as defined above; or reacting a compound of formula I in which n is 0 to 4 with an amino acid or oligopeptide of the formula



III

in which:

n is 1—5;

P is as defined above; and

Z is an amino protective group as known in the peptide art, in the presence of an amido coupling agent

as known in the peptide art, and, optionally in either process, removing any protecting groups and forming a salt of the compound of formula I.

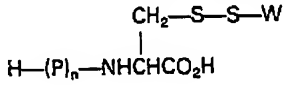
11. A compound of claims 1—9 for pharmaceutical use.

12. A compound of claims 1—9 for anti-microbial use.

13. A pharmaceutical composition which comprises a compound of any one of claims 1—9 and a pharmaceutically acceptable carrier.

Claims for the Contracting State: AT

1. A process for preparing a compound of the structural formula:



I

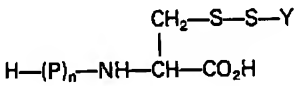
in which:

n is an integer of from 1—5;

P is, individually, alanyl, ornithyl, lysyl or phenylalanyl; and

W is a known residue of an antimicrobial mercaptan, said compound having the L-configuration at the carrying C-unit; or a salt thereof;

comprising either reacting a compound of the structural formula:



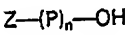
II

in which:

n is an integer of from 0—5;

P is, individually, alanyl, ornithyl, lysyl or phenylalanyl; and

Y is guanyl, pyridyl when W is other than pyridyl or pyridyl-N-oxide when W is other than pyridyl-N-oxide, with a compound of the formula, H—S—W, in which W is as defined above; or reacting a compound of formula I in which n is 0 to 4 with an amino acid or oligopeptide of the formula



III

in which:

n is 1—5;

P is as defined above; and

Z is an amino protective group as known in the peptide art, in the presence of an amido coupling agent as known in the peptide art, and, optionally in either process, removing any protecting groups and forming a salt of the compound of formula I.

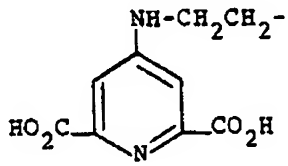
2. A process according to claim 1 for preparing a compound in which the peptide unit adjacent to the carrying C-unit has the L-configuration.

3. A process according to claim 1 for preparing a compound in which P is L-alanyl.

4. A process according to claim 1 for preparing a compound in which P is L-alanyl, n is 1 and W is a pyridyl.

5. A process according to claim 1 for preparing a compound in which P is L-alanyl, n is 1 and W is a 2-pyridyl or 2-pyridyl-N-oxide.

6. A process according to claim 1 for preparing a compound in which P is L-alanyl, n is 2 and W is:



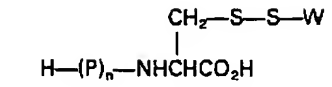
7. A process according to claim 1 for preparing a compound in which P is L-ornithyl and n is 1.

8. A process according to claim 1 for preparing a compound in which H—(P)$_n$— is L-ornithyl-L-alanyl.

Patentansprüche für die Vertragsstaaten: BE CH DE FR GB IT LI LU NL SE

1. Ein Oligopeptid mit einer freien Aminogruppe, das aus 2 bis 6 Einheiten zusammengesetzt ist, die ausgewählt sind aus der Gruppe Alanyl, Ornithyl, Lysyl und Phenylalanyl, und das eine L-Alanyleinheit enthält, die am β-C-Atom mit einer Gruppe —S—S—W substituiert ist, in der W ein bekannter Rest eines antomikrobiell wirksamen Mercaptans ist.

2. Eine Verbindung der Strukturformel



in der n eine ganz Zahl von 1 bis 5 ist,
P entweder Alanyl, Ornithyl, Lysyl- oder Phenylalanyl ist;
und W ein bekannter Rest eines antimikrobiell wirksamen Mercaptans ist, wobei diese Verbindung an der tragenden C-Einheit die L-Konfiguration hat; oder ein Salz davon.

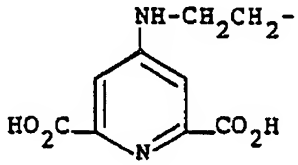
3. Die Verbindung nach Anspruch 2, in der die der tragenden C-Einheit benachbarte Peptideinheit die L-Konfiguration hat.

4. Die Verbindung nach Anspruch 2, in der P L-Alanyl ist.

5. Die Verbindung nach Anspruch 2, in der P Alanyl ist, n den Wert 1 hat und W ein Pyridyl ist.

6. Die Verbindung nach Anspruch 2, in der P L-Alanyl ist, n den Wert 1 hat und W 2-Pyridyl oder 2-Pyridyl-N-oxid ist.

7. Die Verbindung nach Anspruch 2, in der P L-alanyl ist, n den Wert 2 hat und W

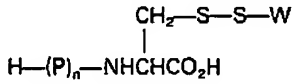


ist.

8. Die Verbindung nach Anspruch 2, in der P L-Ornithyl ist und n den Wert 1 hat.

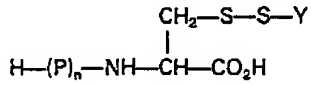
9. Die Verbindung nach Anspruch 2, in der H—(P)$_n$— L-Ornithyl-L-alanyl ist.

10. Verfahren zur Herstellung einer Verbindung der Strukturformel



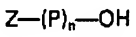
(I)

in der n eine ganz Zahl von 1 bis 5 ist,
P entweder Alanyl, Ornithyl, Lysyl oder Phenylalanyl ist; und
W ein bekannter Rest eines antimikrobiell wirksamen Mercaptans ist, wobei diese Verbindung an der tragenden C-Einheit die L-Konfiguration hat, oder eines Salzes davon, dadurch gekennzeichnet, daß man entweder eine Verbindung der Strukturformel



(II)

in der n eine ganze Zahl von 0 bis 5 ist,
P entweder Alanyl, Ornithyl, Lysyl- oder Phenylalanyl ist, und Y Guanyl ist oder Pyridyl ist, wenn W nicht Pyridyl ist, oder Pyridyl-N-oxid ist, wenn W nicht Pyridyl-N-oxid ist, mit einer Verbindung der formel H—S—W umsetzt, in der W die vorstehend angegebene Bedeutung hat, oder eine Verbindung der Formel I, in der n den Wert 0 bis 4 hat, mit einer Aminosäure oder einem Oligopeptid der Formel



III

in der n den Wert 1 bis 5 hat,
P die vorstehend angegebene Bedeutung hat, und
Z ein auf dem Peptidgebiet bekannte Amino-Schutzgruppe ist, in Gegenwart eines auf dem Peptidgebiet bekannten Amido-Kupplungsmittels umsetzt, und gegebenenfalls vorhandene Schutzgruppen abspaltet und ein Salz der Verbindung der Formel I bildet.

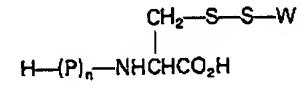
11. Eine Verbindung nach Ansprüche 1 bis 9 zur pharmazeutischen Verwendung.

12. Eine Verbindung nach Ansprüche 1 bis 9 zur antimikrobiellen Verwendung.

13. Arzneimittel, das eine Verbindung nach einem der Ansprüche 1 bis 9 und einen pharmazeutisch verträglichen Träger enthält.

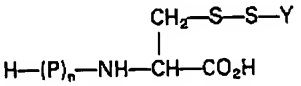
Patentansprüche für den Vertragsstaat: AT

1. Verfahren zur Herstellung einer Verbindung der Strukturformel



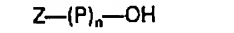
(I)

in der n eine ganz Zahl von 1 bis 5 ist,
P entweder Alanyl, Ornithyl, Lysyl oder Phenylalanyl ist; und
W ein bekannter Rest eines antimikrobiell wirksamen Mercaptans ist, wobei diese Verbindung an der tragenden C-Einheit die L-Konfiguration hat, oder eines Salzes davon, dadurch gekennzeichnet, daß man entweder eine Verbindung der Strukturformel



(II)

in der n eine ganz Zahl von 1 bis 5 ist,
P entweder Alanyl, Ornithyl, Lysyl- oder Phenylalanyl ist, und Y Guanyl ist oder Pyridyl ist, wenn W nicht Pyridyl ist, oder Pyridyl-N-oxid ist, wenn W nicht Pyridyl-N-oxid ist, mit einer Verbindung der formel H—S—W umsetzt, in der W die vorstehend angegebene Bedeutung hat, oder eine Verbindung der Formel I, in der n den Wert 0 bis 4 hat, mit einer Aminosäure oder einem Oligopeptid der Formel



(III)

in der n den Wert 1 bis 5 hat,
P die vorstehend angegebene Bedeutung hat, und
Z ein auf dem Peptidgebiet bekannte Amino-Schutzgruppe ist, in Gegenwart eines auf dem Peptidgebiet bekannten Amido-Kupplungsmittels umsetzt, und gegebenenfalls vorhandene Schutzgruppen abspaltet und ein Salz der Verbindung der Formel I bildet.

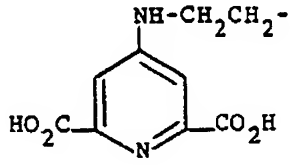
2. Verfahren nach Anspruch 1 zur Herstellung einer Verbindung, in der die der tragenden C-Einheit benachbarte Peptideinheit die L-Konfiguration hat.

3. Verfahren nach Anspruch 1 zur Herstellung einer Verbindung in der P L-Alanyl ist.

4. Verfahren nach Anspruch 1 zur Herstellung einer Verbindung, in der P L-Alanyl ist, n den Wert 1 hat und W ein Pyridyl ist.

5. Verfahren nach Anspruch 1 zur Herstellung einer Verbindung, in der P L-Alanyl ist, n den Wert 1 hat und W entweder ein 2-Pyridyl oder 2-Pyridyl-N-oxid ist.

6. Verfahren nach Anspruch 1 zur Herstellung einer Verbindung, in der P L-Alanyl ist, n den Wert 2 hat und W



ist.

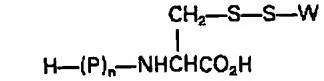
7. Verfahren nach Anspruch 1 zur Herstellung einer Verbindung, in der P L-Ornithyl ist und n den Wert 1 hat.

8. Verfahren nach Anspruch 1 zur Herstellung einer Verbindung, in der H—(P)$_n$— L-Ornithyl-L-alanyl ist.

Revendications pour les Etats contractants: BE CH DE FR GB IT LI LU NL SE

1. Une Oligopeptide à 2—6 unités (contenant un groupe amino libre) formé d'unités sélectionnées à partir d'alanyl, d'ornithyl, de lysyl et de phénylalanyl, qui conprend une unité L-alanyl β-substituée avec un groupe —S—S—W dans lequel W est un résidu connu de mercaptan anti-microbien.

2. Un composé de la formule structurelle:



dans laquelle:
n est un nombre entier entre 1. et 5;
P est, individuellement, alanyl, ornithyl, lysyl ou phénylalanyl; et

W est un résidu connu de mercaptan anti-microbien, ledit composé ayant la configuration L à l'unité porteuse C; ou un sel du même.

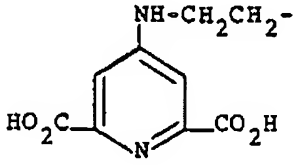
3. Le composé de la revendication 2, dans laquelle l'unité peptide adjacente à l'unité porteuse C a la configuration L.

4. Le composé de la revendication 2 dans laquelle P est L-alanyl.

5. Le composé de la revendication 2 dans laquelle P est L-alanyl, n est 1 et W est un pyridyl.

6. Le composé de la revendication 2 dans laquelle P est L-alanyl, n est 1 et W est 2-pyridyl ou 2-pyridyl-N-oxyde.

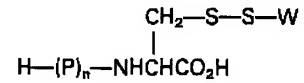
7. Le composé de la revendication 2, dans laquelle P est L-alanyl, n est 2 et W est:



8. Le composé de la revendication 2 dans laquelle P est L-ornithyl et n est 1.

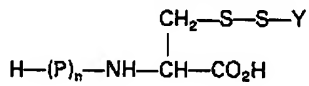
9. Le composé de la revendication 2 dans laquelle H-$(P)_n$— est L-ornithyl-L-alanyl.

10. Un procédé pour la préparation d'un composé de la formule structurelle:



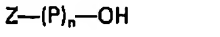
I

dans laquelle:
n est un nombre entier entre 1 et 5;
P est, individuellement, alanyl, ornithyl, lysyl ou phénylalanyl; et
W est un résidu connu d'un mercaptan anti-microbien, ledit composé ayant la configuration L à l'unité porteuse C; ou un sel du même.
comprenant soit la réaction d'un composé de la formule structurelle:



II

dans laquelle:
n est un nombre entier entre 0 et 5;
P est, individuellement, alanyl, ornithyl, lysyl, ou phénylalanyl; et
Y est guanyl, pyridyl, lorsque W est autre que pyridyl ou pyridyl-N-oxyde lorsque W est autre que pyridyl-N-oxyde, avec un composé de la formule H—S—W, dans laquelle W est comme défini ci-dessus; soit la réaction d'un composé de la formule I dans laquelle n est entre 0 et 4 avec un acide amino ou un oligopeptide de la formule



III

dans laquelle:
n est entre 1 et 5;
P est comme défini ci-dessus; et
Z est un groupe protecteur amino comme il est connu dans l'art peptide, en présence d'un agent d'accouplement amido comme il est connu dans l'art peptide, et, de manière facultative, dans l'un ou l'autre des procédés, en éliminant tout groupe protecteur et en formant un sel du composé de la formule I.

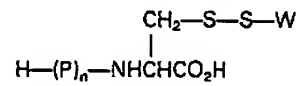
11. Un composé des revendications de 1 à 9 à usage pharmaceutique.

12. Un composé des revendications de 1 à 9 à usage anti-microbien.

13. Une composition pharmaceutique qui comprend un composé de n'importe laquelle des revendications de 1 à 9 et un support pharmaceutique acceptable.

Revendications pour l'Etat contractant: AT

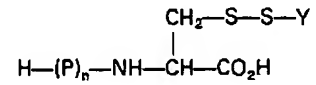
1. Un procédé pour la préparation d'un composé de la formule structurelle:



I

dans laquelle:
n est un nombre entier entre 1 et 5;
P est, individuellement, alanyl, ornithyl, lysyl ou phénylalanyl; et

W est un résidu connu d'un mercaptan anti-microbien, ledit composé ayant la configuration L à l'unité C porteuse, ou un sel du même;
comprenant soit la réaction d'un composé de la formule structurelle:



II

dans laquelle:
n est un nombre entier de 0 et 5;
P est, individuellement, alanyl, ornithyl, lysyl, ou phénylalanyl; et
Y est guanyl, pyridyl, lorsque W est autre que pyridyl ou pyridyl-N-oxyde lorsque W est autre que pyridyl-N-oxyde, avec un composé de la formule, H—S—W, dans laquelle W est comme défini ci-dessus; soit la réaction d'un composé de la formule I dans laquelle n est de 0 à 4 avec un acide amino ou un oligopeptide de la formule

III

dans laquelle:
n est entre 1 et 5;
P est comme défini plus haut; et
Z est un groupe protecteur amino comme il est connu dans l'art peptide, en présence d'un agent d'accouplement amido comme il est connu dans l'art peptide, et de manière facultative dans n'importe lequel des procédés, en éliminant tout groupe protecteur et en formant un sel du composé de la formule I.

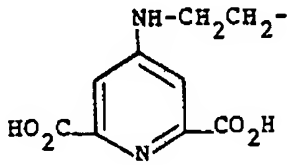
2. Un procédé selon la revendication 1 pour la préparation d'un composé dans lequel l'unité peptide adjacente à l'unité C porteuse a la configuration L.

3. Un procédé selon la revendication 1 pour la préparation d'un composé dans lequel P est L-alanyl.

4. Un procédé selon la revendication 1 pour la préparation d'un composé dans lequel P est L-alanyl, n est 1 et W est un pyridyl.

5. Un procédé selon la revendication 1 pour la préparation d'un composé dans lequel P est L-alanyl, n est 1 et W est 2-pyridyl ou 2-pyridyl-N-oxyde.

6. Un procédé selon la revendication 1 pour la préparation d'un composé dans lequel P est L-alanyl, n est 2 et W est:



7. Un procédé selon la revendication 1 pour la préparation d'un composé dans lequel P est L-ornithyl et n est 1.

8. Un procédé selon la revendication 1 pour la préparation d'un composé dans lequel H—(P)$_n$— est L-ornithyl-L-alanyl.